

Staphylococcus aureus Cas9

an alternative Cas9 for genome editing applications

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Summary

S. aureus Cas9:

- Is encoded by 3159 nucleotides, compared to 4104 nucleotides for *S. pyogenes* Cas9.
- Recognizes an NNGRRT PAM.
- Cleaves dsDNA with efficiency comparable to *S. pyogenes* Cas9.
- Can be packaged into AAV with U6:gRNA
- Cleaves dsDNA with gRNAs of different spacer lengths, with guides shorter than 20 generally less efficient.
- D10A and N580A *S. aureus* nickases are active

Methods: All non-AAV experiments were performed via transfection into HEK293FT cells using Lipofectamine 3000 or Mirus in 24-well format. Transfections consisted of 750 ng/well of Cas9 (driven by CMV promoter) plasmid and 250 ng/well of gRNA (driven by U6 promoter) construct. Total DNA was harvested from cells 72 hours post transfection, and % NHEJ was determined by target locus PCR followed by T7E1 cleavage assay.

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S. aureus Cas9 cleavage efficiency is comparable to S. pyogenes Cas9

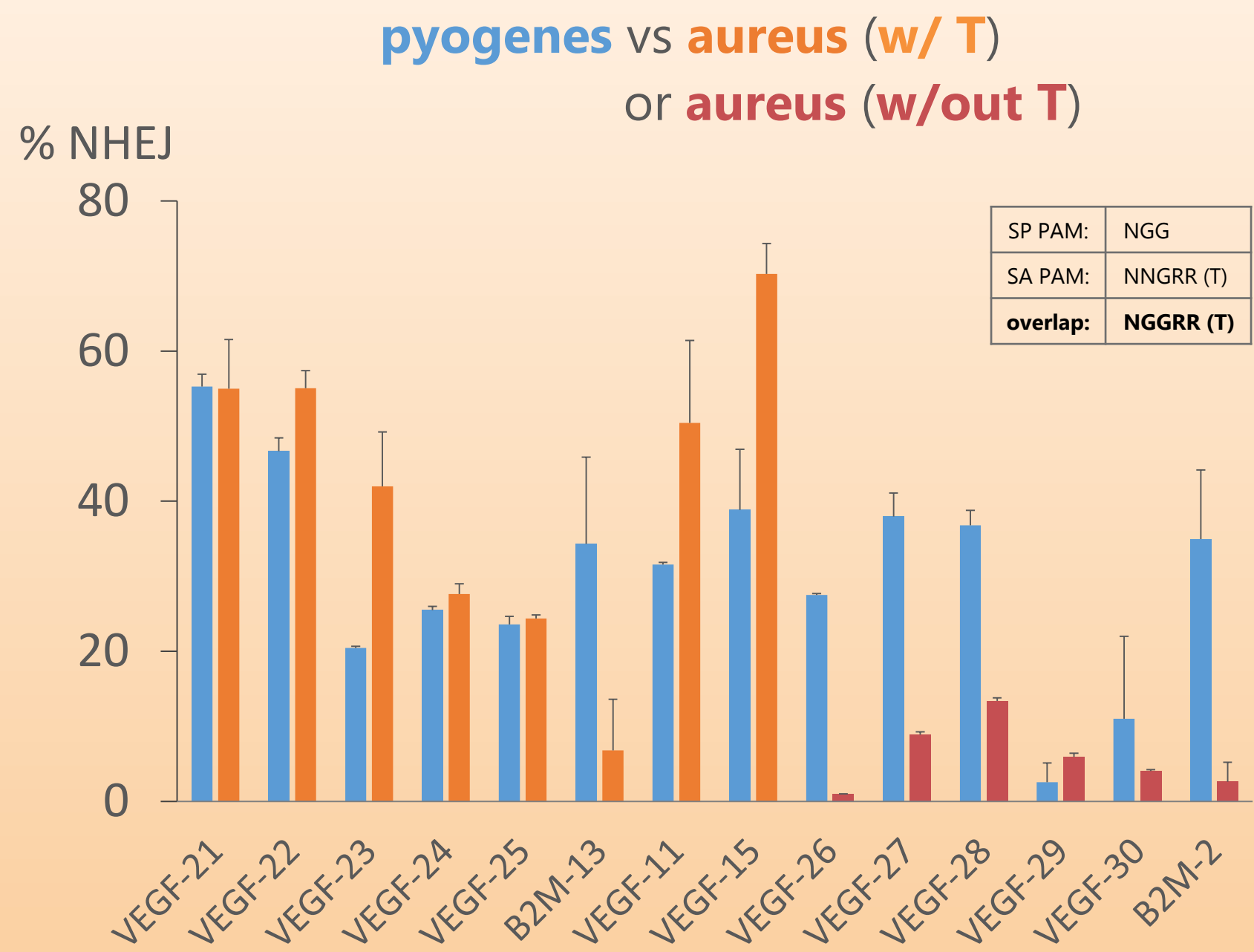
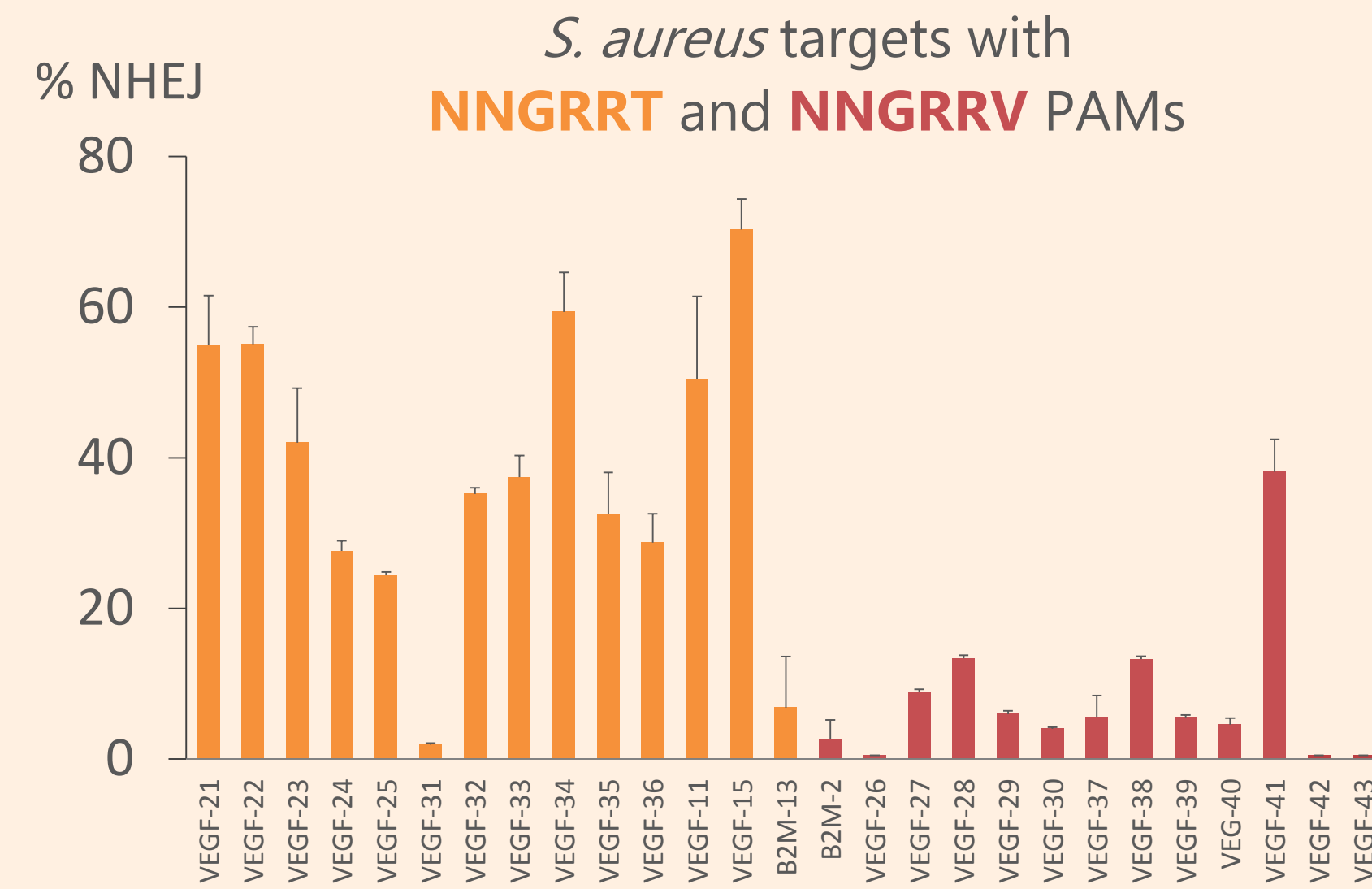
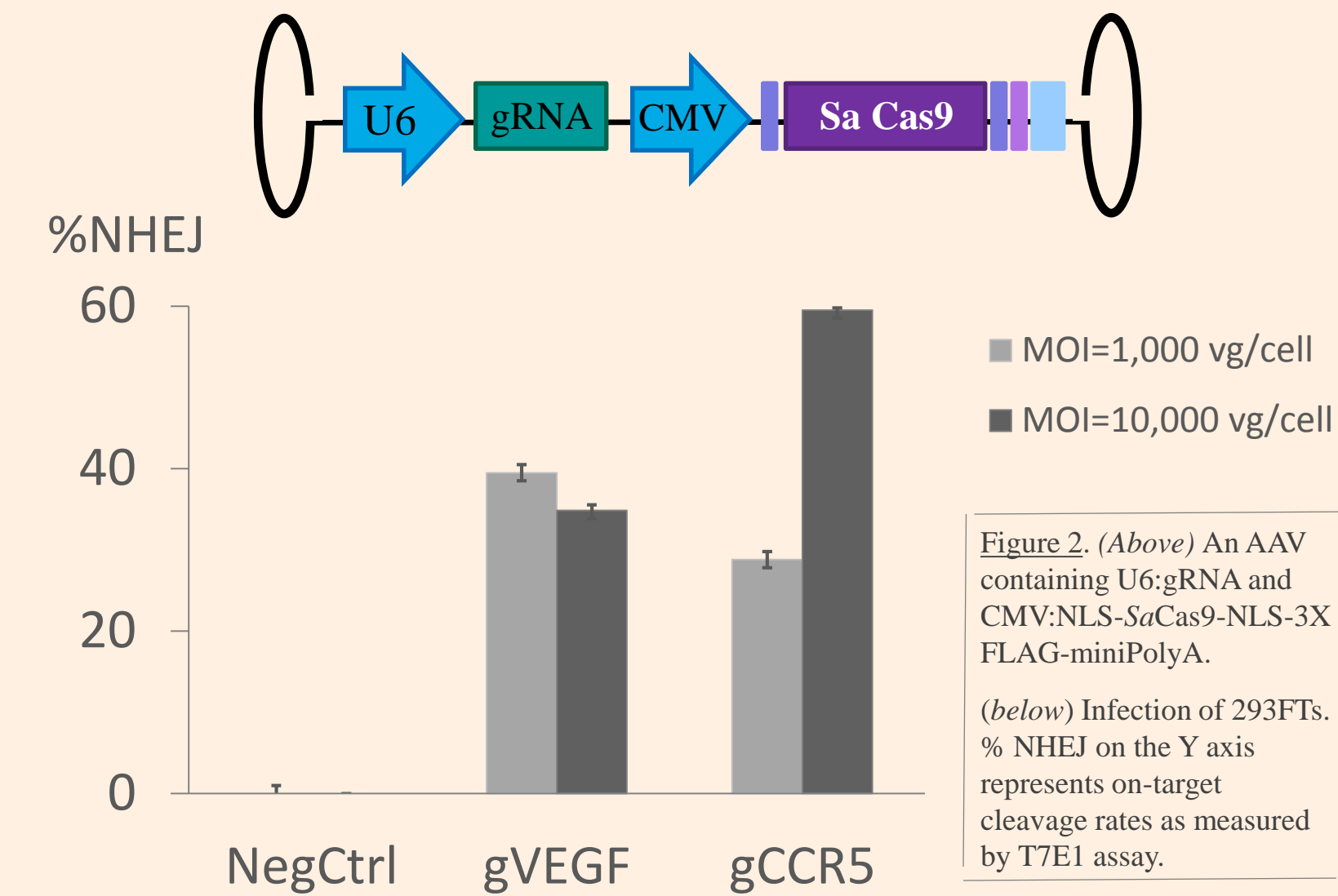


Figure 1. (Above) *S. aureus* Cas9 with gRNAs targeting loci with different PAMs. % NHEJ on the Y axis represents on-target cleavage rates as measured by T7E1 assay. (Below) Comparison of *S. aureus* Cas9 and *S. pyogenes* Cas9 DNA cleavage at identical target sites, with dual-compatible NNGRR(T) PAMs.

AAV delivery of Sa Cas9 and gRNA



Cleavage efficiency depends on gRNA spacer length

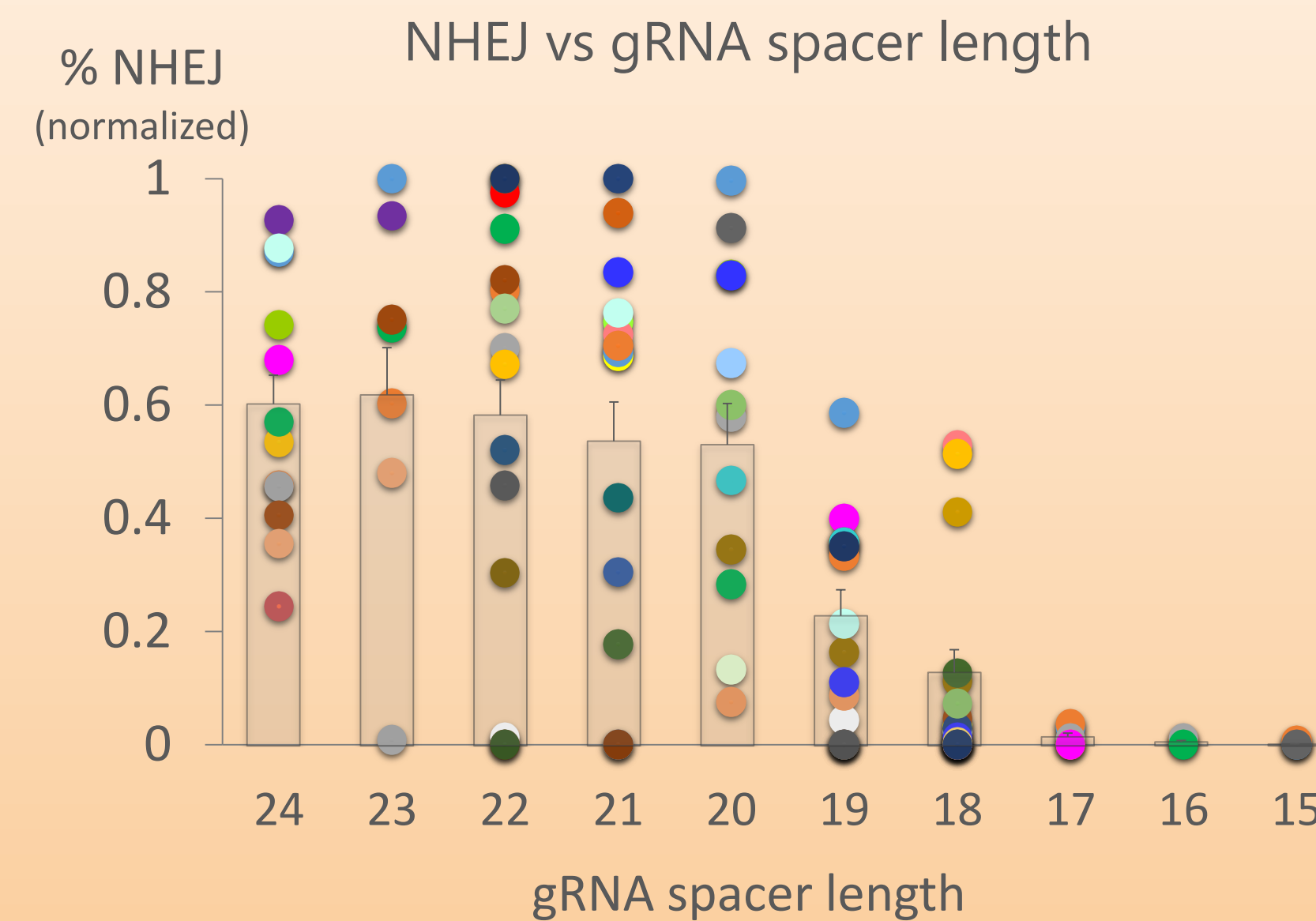


Figure 3. *S. aureus* Cas9 cleavage of target DNA with "sibling" gRNAs, which have spacers of different lengths but target the same precise locus. Same-colored dots represent gRNA siblings, all of which initiate with a G for optimal U6 promoter expression. Data points come from multiple experiments, with maximum cleavage in each experiment set to 1 and all other data points normalized. Grey bars represent mean cleavage for gRNAs of that length.

S. aureus Cas9 nickases D10A and N580A

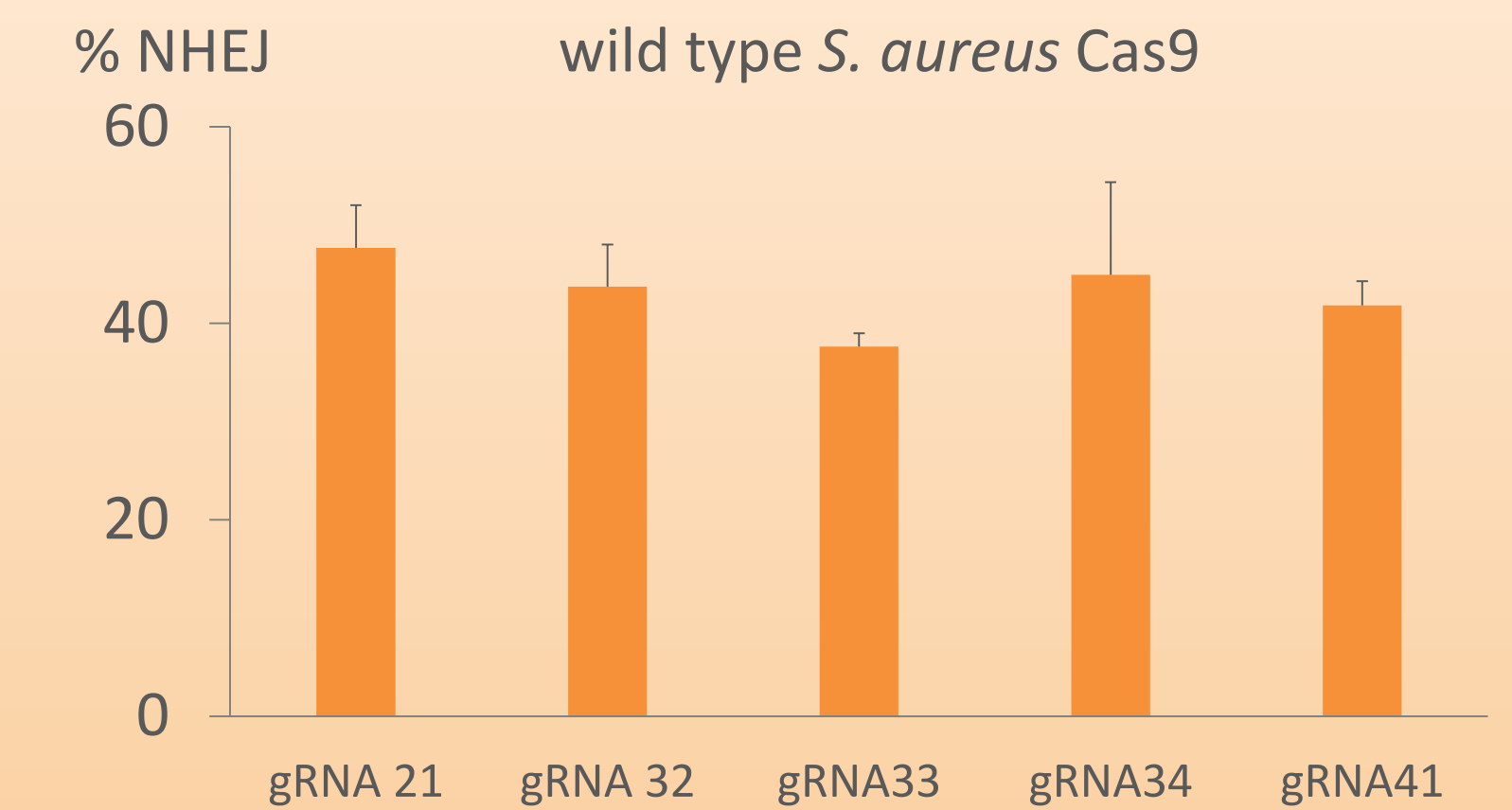
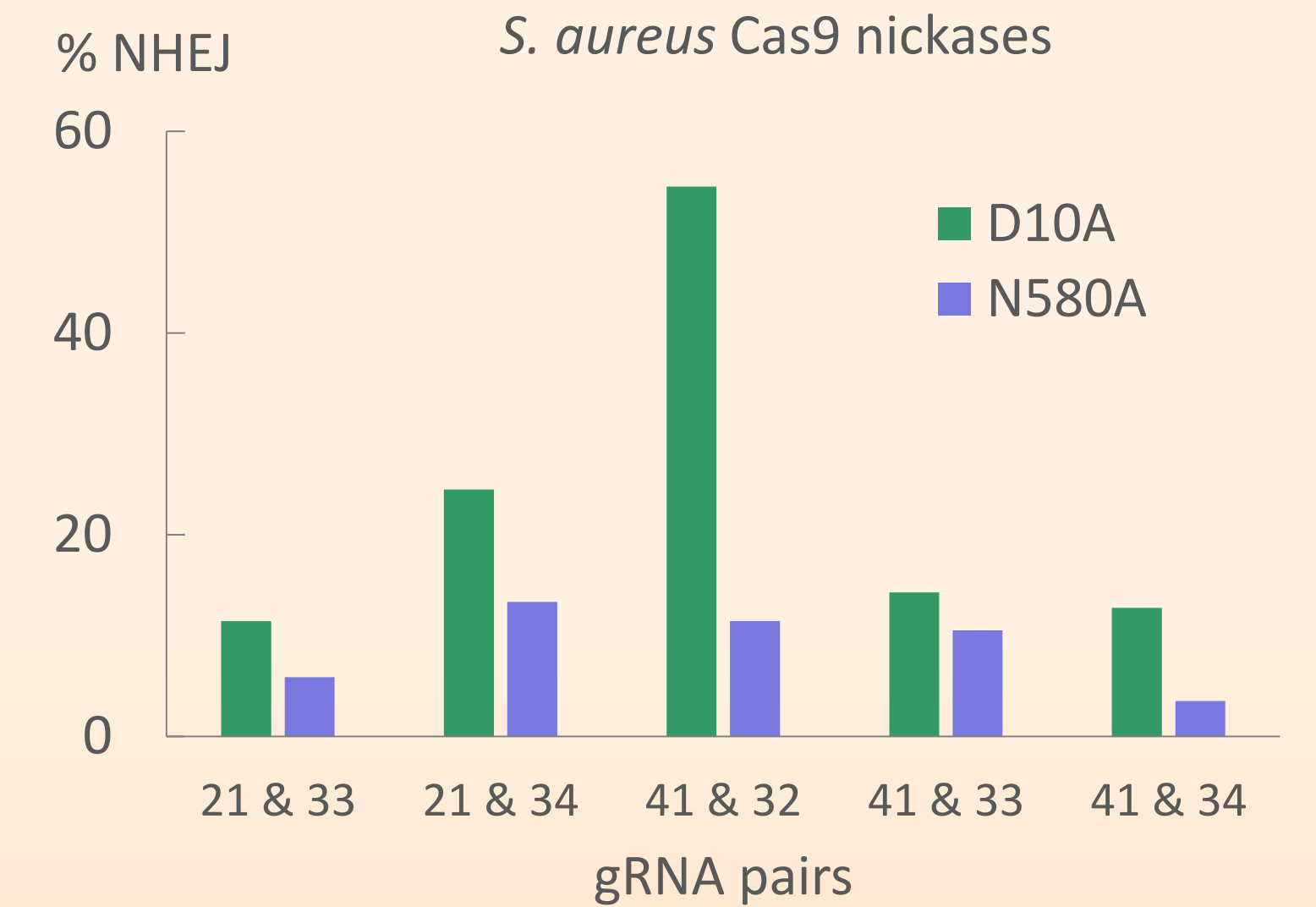
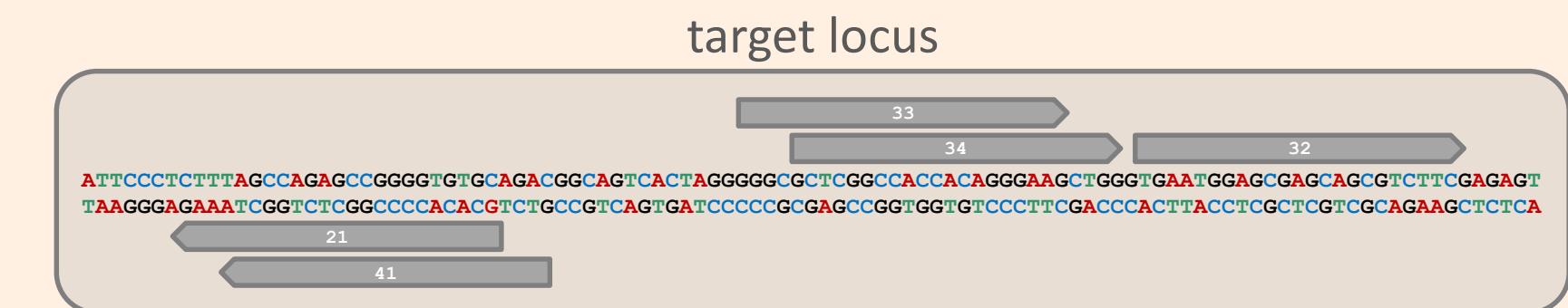


Figure 4. (Above, top) Sequence of the target locus with five gRNAs annotated in grey. (Above, mid) D10A and N580A *S. aureus* Cas9 nickases cleaving endogenous loci using pairs of gRNAs. NHEJ % on the Y axis represents on-target cleavage rates as measured by TOPO sequencing. (Above, bottom) Wild type *S. aureus* Cas9 with gRNAs targeting five different loci. NHEJ % on the Y axis represents on-target cleavage rates as measured by T7E1 assay.